PROTEOLYTIC ACTIVITY CHARACTERIZATION OF BACTERIA ISOLATED FROM MALAYSIAN TRADITIONAL FERMENTED FOOD

NAJIHAH BINTI ISMAIL

A dissertation submitted in partial fulfilment of the
requirements for the award of the degree of
Master of Science (Biotechnology)

Faculty of Biosciences and Medical Engineering
Universiti Teknologi Malaysia

JULY 2014

سُبْحَانَ اللّٰهِ وَ بِحَمْدِهِ ، سُبْحَانَ اللّٰهِ الْعَظِيمِ

"*Maha Suci ALLAH & segala puji bagi-NYA, Maha Suci ALLAH yang Maha Agung"*

To heart of my life Ma, Abah, Abg Zi, Q. Ngah, Q. Chik, Q. Jue, & Pok Pi

To my beloved fiancé Mohd Saiful bin Deraman

To all my awesome friends

ACKNOWLEDGEMENT

Bismillahirrahmanirrahim, Alhamdulillah. First and foremost I would like to highly thank my supervisor Dr. Haryati Jamaluddin for giving me this golden opportunity to perform this project and for remarkably supervised me throughout this project duration. I truthfully appreciated all the valuable knowledge, moral support and advise that had been given. My innermost gratitude to my beloved parents *Ma* and *Abah*, and also my siblings, *Abg*, *Kak Ngah*, *Kak Ju* and *Pok Pi* for always be at my side through the hardship of completing this project and for encourage me to do my best in this project. I would also like to specially thank my late beloved sister, *Kak Chik*, for giving me such a precious advise, grow wonderful spirit in me and for believing in me (al-fatihah).

Besides, I would like to earnestly thank to all postgraduate student of Biological Structure Laboratory, staffs and laboratory assistants for kindly and helpfully helping me in completing my project. In addition, millions thank to my awesome friends *Nad*, *Kak Dalila*, *Zara Kak Lin*, *Soraya* and all my MQT course mates for thoughtfully sharing important knowledge with me, for being together through thick and thin and for making performing this project such a wonderful and memorable experience. Last but not least, special thanks to *Mohd Saiful bin Deraman* for always be there.

ABSTRACT

Three types of bacteria strains, which are *Bacillus* sp., *Enterococcus gallinarum* and *Bacillus thuringiensis* have been isolated previously from Malaysian traditional fermented food. The proteolytic activities of the three strains were screened on skim milk agar plate. After 24 hours of incubation at 37°C, proteolytic activity was observed based on holozone formation on the skim milk agar plate with a diameter of 0, 1.9, and 3.2 cm respectively for *Bacillus* sp., *Enterococcus gallinarum* and *Bacillus thuringiensis*. The proteolytic activities of all the strains were characterized based on optimum temperature, temperature stability, optimum pH, pH stability, substrate specificity and effect of metal ions towards activity. All three strains showed optimum activity at 50°C. The optimum pH for *Bacillus* sp. and *Bacillus thuringiensis* were pH 8.5, while *Enterococcus gallinarum* showed maximum enzyme activity of 0.068±0.003 U/ml at pH 7.5. The proteolytic activity of the bacteria were stable in the temperature range of 30°C to 50°C and exhibited rapid decrease in activity when incubated at 60°C for 60 minutes. Proteolytic activity of all strains was stable at a broad pH range from pH 4.5 to pH 10.6. The bacteria strains displayed high activity for casein, gelatin and fibrin but showed very low activity for bovine serum albumin. Proteolytic activity of *Bacillus* sp. was enhanced by Cu^{2+}, Ca^{2+}, Mg^{2+}, Fe^{2+} and Zn^{2+}, while the proteolytic activity of *Enterococcus gallinarum* was only slightly enhanced by Zn^{2+}.

ABSTRAK

Tiga jenis spesis bakteria daripada makanan terampai tradisional Malaysia telah dipencilkan iaitu *Bacillus* sp., *Enterococcus gallinarum* dan *Bacillus thuringiensis*. Aktiviti proteolitik oleh ketiga-tiga spesis bakteria telah ditentukan menggunakan plat agar susu skim. Selepas inkubasi selama 24 jam pada suhu 37°C, aktiviti proteolitik dapat dilihat melalui pembentukkan kawasan jernih pada plat agar susu skim dengan masing-masing mempunyai diameter (dalam cm) iaitu 0. 1.9, dan 3.2 bagi *Bacillus* sp., *Enterococcus gallinarum,* dan *Bacillusthuringiensis*. Aktiviti proteolitik olehsetiap spesis bakteria dicirikan mengikut suhu optimum, kestabilan suhu, pH optimum, kestabilan pH, pengkhususan substrat dan juga kesan ion logam terhadap aktiviti proteolitik. Setiap spesis bakteria menunjukkan aktiviti optima pada suhu 50°C. pH optima bagi *Bacillus* sp. dan *Bacillus thuringiensis* adalah pH 8.5, manakala *Enterococcus gallinarum* menunjukkan aktiviti enzim yang maksimum iaitu 0.068±0.003 U/ml pada pH 7.5. Aktiviti proteolitik oleh setiap bakteria adalah stabil di dalam linkungan suhu 30°C hingga 50°C dan menurun secara mendadak selepas inkubasi pada suhu 60°C selama 60 minit. Aktiviti proteolitik bagi setiap spesis bakteria adalah stabil dalam lingkungan pH yang luas iaitu daripada pH 4.5 hingga pH 10.6. Ketiga-tiga spesis bakteria menunjukkan aktiviti yang tinggi bagi casein, gelatin dan fibrin tetapi menunjukkan aktiviti yang rendah bagi albumin serum bovin (BSA). Aktiviti proteolitik bagi *Bacillus* sp. dipertingkatkan oleh Cu^{2+}, Ca^{2+}, Mg^{2+}, Fe^{2+}dan Zn^{2+}. Manakala aktiviti proteolitik bagi *Enterococcus gallinarum* hanya dipertingkat sedikit oleh Zn^{2+}.